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Effects of n-3 fatty acids on renal function and renal prostaglandin E metabolism

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Effects of n-3 fatty acids on renal function and renal prostaglandin E metabolism. The present study was performed to investigate the effects of dietary fish oil supplements on renal function and renal prostaglandin (PG) E metabolism. The usual "western" diet of 10 healthy volunteers (six female and 4 male) aged between 21 and 35 years was supplemented with 6 g/day of n-3 polyunsaturated fatty acids [3.6 g of eicosapentaenoic acid (EPA) and 2.4 g of docosahexaenoic acid (DHA)] for six weeks. Supine arterial blood pressure (BP) and heart rate (HR), renal hemodynamics, renal excretory function and urinary excretion of PGE_2 and PGE_3 were determined before and at the end of the fish oil supplementation period. No changes could be observed in BP and HR while renal plasma flow (RPF), determined as the clearance of PAH, significantly increased from 559 ± 44 to 738 ± 47 ml/min ($P < 0.001$) with the fish oil supplements. This was associated with a decrease in renal vascular resistance from $(8.11 \pm 0.54) \cdot 10^{-2}$ to $(6.37 \pm 0.38) \cdot 10^{-2}$ mm Hg · min · ml^{-1} ($P < 0.01$). Glomerular filtration rate (GFR), determined as the clearance of inulin, increased from 97 ± 3 to 107 ± 3 ml/min ($P < 0.01$), resulting in a decrease in filtration fraction from an average of 0.19 ± 0.01 to 0.15 ± 0.01 ($P < 0.01$). This was paralleled by an increase in urine volume (V) (13.8 ± 0.9 vs. 12.2 ± 1.0 ml/min; $P < 0.05$), urinary excretion of inorganic phosphate ($U_{Phos}V$; 20.6 ± 2.0 vs. 16.9 ± 2.0 μmol/min; $P < 0.05$) and C_{H_2O} (9.6 ± 0.6 vs. 8.5 ± 0.7 ml/min) in the presence of unchanged $U_{Na}V$, U_KV, $U_{Cl}V$, C_{Osm} and C_{H_2O}/GFR determined during maximal water diuresis and hypotonic (0.45%) saline infusion. Urinary excretion of PGE_2 determined in the six female participants decreased with the fish oil supplements (168.0 ± 13.0 vs. 125.3 ± 11.8 ng/24 hr; $P < 0.01$), while urinary excretion of PGE_3 averaged 6.9 ± 0.8 ng/24 hr during control and increased to 23.4 ± 3.2 ng/24 hr ($P < 0.01$) with the n-3 fatty acid supplements. Our study demonstrates that supplementation of a regular "western" diet with EPA and DHA in the here chosen doses over six weeks markedly affects renal hemodynamics, with increases in RPF and GFR. In spite of an increased filtered load, unchanged electrolyte excretion implies increased tubular reabsorption which may, at least in part, be located in the diluting segments of the nephron. These renal hemodynamic and tubular functional changes are associated with quantitative and qualitative alterations in renal prostanoid metabolism. However, the precise interrelationship between the alterations in renal eicosanoid metabolism and the changes observed in renal hemodynamics and renal excretory function remains to be established.

n-3 (also called omega-3) fatty acids are polyunsaturated fatty acids with a double bond between the third and fourth carbon atom from the methyl end of the acid. These fatty acids are scarce in a normal "western" diet, but make up a significant portion of the fat in cold water fish [1]. Such fish oil supplements have been shown to affect a variety of cardiovascular functions. Thus, a decrease in arterial blood pressure and/or a blunted response to the administration of exogenous angiotensin II have been reported [2–8]. The mechanisms of the cardiovascular effects of n-3 fatty acid supplements remain unclear. Since prostaglandins, prostacyclin, thromboxane, leukotrienes and other eicosanoids may be important modulators within the cardiovascular system [9–11], speculations center around changes in eicosanoid metabolism induced by the dietary intervention as mediators of the observed changes.

In addition to the proposed effects of n-3 fatty acids within the systemic circulation, detailed information about their effects on renal hemodynamics in humans is not available to date. Moreover, only scarce data exist on the effects of dietary n-3 fatty acids supplements on renal prostaglandin E (PGE) metabolism [12]. Lastly, it is at present unknown whether fish oil supplements affect renal excretory function. Such an effect could be mediated, among others, by alterations in renal hemodynamics and/or renal PGE metabolism.

In the present study, we have therefore investigated the effects of a six week supplementation period with eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) on renal hemodynamics, PGE metabolism and excretory function in normal volunteer subjects.

Methods

The study was performed in 10 healthy volunteers (six female and four male subjects), aged between 21 and 35 years, who were on a regular "western" diet containing approximately 160 mmol of sodium and 80 mmol of potassium/day. All volunteers were studied twice, first during a control period and then again following six weeks of dietary fish oil supplements. n-3 fatty acids were administered as capsules (MaxEPA, Hull, UK) given p.o. (20 capsules/day in divided doses) yielding doses of 3.6 g of eicosapentaenoic acid (EPA) and 2.4 g of docosahexaenoic acid (DHA)/day. To further document compliance to the study protocol, platelet aggregation was determined before and at the end of the dietary supplementation period. In all ten volunteers, platelet aggregation following ADP, 1 μmol/ml of platelet rich plasma (PRP), or collagen, 1 μg/ml PRP, was suppressed by an average of 18 and 16%, respectively, which is

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an expected effect following dietary fish oil supplementation [13–15].

Arterial blood pressure was measured in the supine position following a 30 minute rest, by using a Dinamap (1846 SX) device over 30 minutes at one-minute intervals. Heart rates were taken concomitantly using continuous ECG monitoring. Only the values obtained during the last 15 minutes were averaged to allow the volunteers to sufficiently adapt to the blood pressure taking procedure.

Renal hemodynamics and renal excretory function were assessed before and at the end of the n-3 fatty acid supplementation period. After overnight fasting, all subjects received 20 ml/kg body weight of water between 07.00 and 09.00 hours. This was followed by a continuous infusion of hypotonic (0.45%; 77 mmol/kg) saline at a rate of 0.25 ml · min^{-1} · kg^{-1} body weight from 09.00 until 11.00 hours. Urine was collected by voluntary voiding during three 30-minute clearance periods from 09.30 to 10.00, 10.00 to 10.30, and 10.30 to 11.00 hours, respectively. Venous blood for the determination of hematocrit (Hct), serum chloride (Cl), PAH and inulin concentrations and serum osmolality was drawn through an indwelling catheter in an anticubital vein at the midpoint of each clearance period. Glomerular filtration rate (GFR) was measured using the clearance of intravenously administered inulin with a 5 g bolus, followed by continuous infusion of a 2% inulin solution in a dose of 3 ml/min. Renal plasma flow (RPF) was calculated as the clearance of PAH. Using a 20% PAH solution, a saturation dose of 10 ml was administered, followed by a maintainance dose of 0.1 ml/min which was given throughout the protocol. Renal blood flow (RBF) was calculated as $[RPF/(10^2 - Hct)] \cdot 10^2$.

Filtration fraction (FF) was calculated as the quotient of GFR and RPF for each clearance period. Renal vascular resistance (RVR) was estimated as the quotient of mean arterial blood pressure and RBF and expressed as mm Hg · min · ml^{-1}.

Clearance of free water (C_{H_2O}) was calculated by the formula

$$C_{H_2O} = \dot{V} - U_{Osm}V/S_{Osm}$$

where $\dot{V}$ = urine flow, U_{Osm} = urinary osmolality and S_{Osm} = serum osmolality. The clearance of chloride (C_{Cl}) was calculated by the formula

$$C_{Cl} = U_{Cl}V/S_{Cl}$$

where U_{Cl} = urinary chloride concentration and S_{Cl} = serum chloride concentration. Delivery of chloride beyond the proximal nephron to the distal tubules (distal delivery) and distal fractional chloride absorption (DFA_{Cl}) were estimated by clearance methods and calculated as $[(C_{H_2O} + C_{Cl})/GFR] \cdot 10^2$ and $[C_{H_2O}/(C_{H_2O} + C_{Cl})]$, respectively. Thus, distal delivery is expressed as the sum of the fraction of filtered chloride excreted (C_{Cl}/GFR) plus the fractional free water clearance (C_{H_2O}/GFR), while the rate of generation of solute-free water (C_{H_2O}) factored by the amount of chloride delivered to the diluting segments ($C_{H_2O} + C_{Cl}$) is used to estimate DFA_{Cl}. It should be emphasized that the assessment of tubular function with these indirect clearance techniques depends on several assumptions which have been described in detail elsewhere [16]. Of critical importance is the requirement that the formation of solute-free water accurately represents distal tubular NaCl absorption. Therefore, in the present study, experiments were performed under conditions of maximal water diuresis and hypotonic volume expansion to suppress the release of antidiuretic hormone. However, since water movement in the distal nephron may occur even in the absence of antidiuretic hormone [17], it is obvious that both clearance terms can only be approximations of the delivery into the distal nephron and distal tubular reabsorptive capacity.

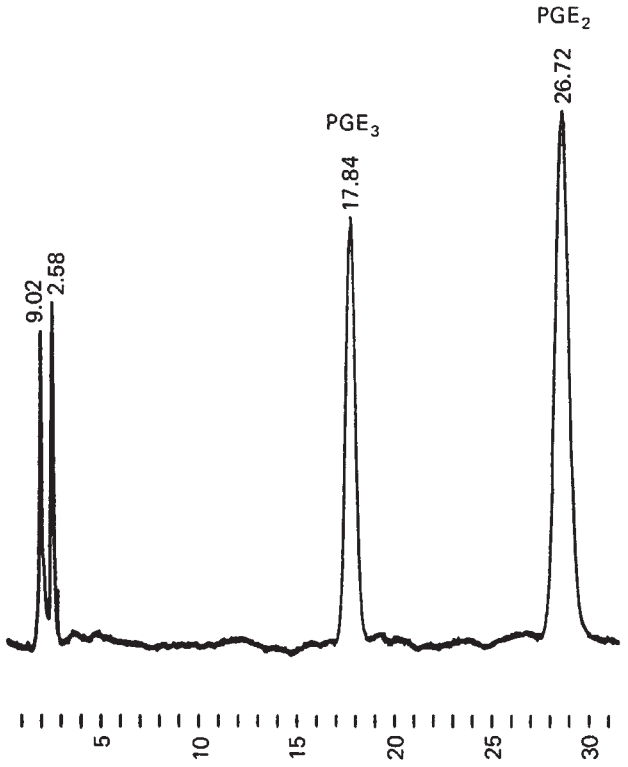

Fig. 1. *HPLC separation of PGE_2 and PGE_3.* Acetonitril/0.02 M KH_2PO_4; pH 3.95; 28/72; wave length 194 nm; column: Lichrosorp RP-18 (see **Methods** for details).

Serum and urinary sodium and potassium concentrations were determined by internal standard flame photometry, serum and urinary chloride concentrations with a Corning chloride meter. Serum and urinary concentrations of inulin, PAH and inorganic phosphate were measured according to established photometric methods. Serum and urinary osmolalities were determined with a Knauer osmometer.

Twenty-four hour urines for the determination of immunoreactive PGE_2 and PGE_3 were collected before and at the end of the dietary supplementation period and were stored frozen until assay. Ten milliliter samples were acidified with citric acid to pH 3.5 and extracted with 4 volumes of ethyl acetate. After evaporation with nitrogen the dry residue was redissolved in 3 ml of H_2O also adjusted to pH 3.5 and passed through a C_{18} cartrige preconditioned with 20 ml of methanol and acidified H_2O. The cartriges were rinsed with 20 ml of H_2O, 20 ml of 10% ethanol and 10 ml petrolether and prostaglandins were then eluted with 20 ml ethyl acetate. The eluate was again dried under nitrogen, dissolved in 0.5 ml H_2O:acetonitrile (72:28) and subjected to HPLC. PGE_2 and PGE_3 were eluted isocratically with 0.02 M KH_2PO_4 (acidified with phosphoric acid to pH 3.95):acetonitrile (72:28). Solvent flow was 1 ml/min (Fig. 1).

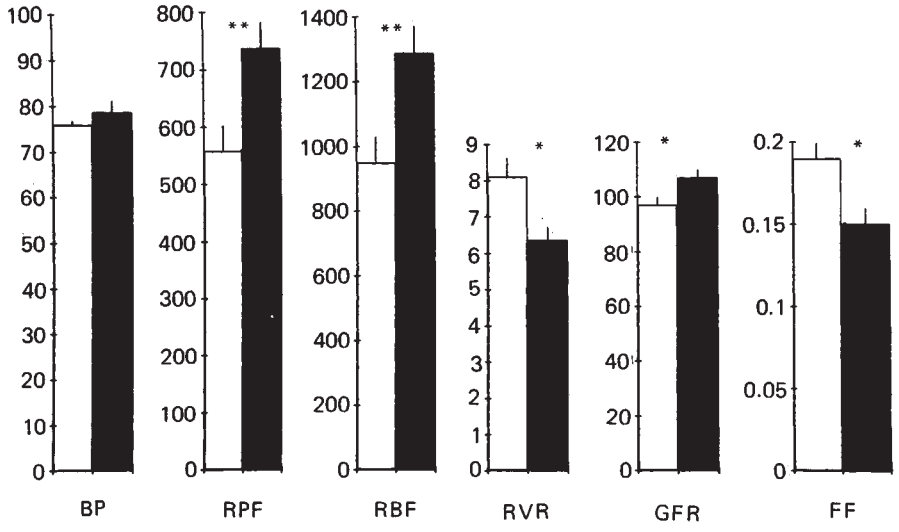

Fig. 2. *Effect of the n-3 fatty acid supplements on mean arterial blood pressure (BP), renal plasma flow (RPF), renal blood flow (RBF), renal vascular resistance (RVR), glomerular filtration rate (GFR), and filtration fraction (FF).* Given are the means ± SEM of the control period (□) and following fish oil supplementation (■).

Following evaporation with nitrogen, the fractions were suspended in buffer and 100 μl portions used for radioimmunoassay. All assays were performed in duplicate using the ^{125}J-PGE_2-RIA kit from New England Nuclear (Dreieich, FRG). PGE_3 was purchased from Cayman Chemical (Ann Arbor, Michigan, USA). This assay system exhibits significant cross-reactivity with PGE_3. Thus, in our assay system, 50% displacement was reached with 2.9 pg of PGE_2 and 8.6 pg of PGE_3. Individual measurements of PGE_2 were corrected for recoveries which were determined by the addition of ^{3}H-PGE_2 into the urine samples. PGE_3 results were corrected by the mean recovery rate of PGE_3 (74.7 ± 3.8%) which was determined by adding known amounts of cold PGE_3 into urine samples.

Results are given as means ± SEM. Statistical analysis was performed using the Wilcoxon matched-pairs signed rank test [18].

Results

The fish oil capsules in the here given dose were tolerated well except for a fishy taste experienced by all but two volunteers. No adverse effects other than this could be noted.

Supine systolic and diastolic arterial BP during control was 110.6 ± 1.9/60.1 ± 1.3 mm Hg with an average mean pressure of 75.9 ± 1.1 mm Hg and a mean heart rate of 69.2 ± 2.8 min^{-1}. These parameters were found unchanged at the end of the dietary intervention period and measured 110.7 ± 2.4/63.5 ± 2.9 mm Hg, 78.7 ± 2.7 mm Hg, and 68.1 ± 3.5 min^{-1}, respectively.

The effect of the n-3 fatty acid supplementation on mean arterial BP, RPF, RBF, RVR, GFR, and FF is illustrated in Figure 2. RPF measured 559 ± 45 ml/min during control and significantly increased by an average of 32% to 738 ± 47 ml/min ($P < 0.001$) following the n-3 fatty acid supplements. Since hematocrit remained unchanged, RBF paralleled these changes in RPF and measured 950 ± 82 and 1288 ± 86 ml/min ($P < 0.001$), respectively. In the presence of unchanged arterial BP, calculated RVR had thus decreased from an average of (8.11 ± 0.54) · 10^{-2} to (6.37 ± 0.38) · 10^{-2} mm Hg · min · ml^{-1} ($P < 0.01$). GFR increased by an average of 10%, from 97 ± 3 to 107 ± 3 ml/min ($P < 0.01$). The proportionally greater increase in RPF as compared to GFR resulted in a fall in filtration fraction from an average of 0.19 ± 0.01 to 0.15 ± 0.01 ($P < 0.01$).

The effect of the n-3 fatty acid supplements on renal excretory function during the three clearance periods is summarized in Table 1. During the control period, urinary excretion of Na^+ ($U_{Na}V$), K^+ (U_KV), and Cl^- ($U_{Cl}V$) averaged 284 ± 50, 132 ± 24, and 224 ± 56 μmol/min, respectively. The dietary intervention had no effect on these renal excretory parameters, which measured 275 ± 36 ($U_{Na}V$), 152 ± 18 (U_KV), and 201 ± 30 μmol/min ($U_{Cl}V$), respectively. Consequently, osmolar clearance was also unchanged by the dietary intervention (4.3 ± 0.5 vs. 4.4 ± 0.3 ml/min). Urine volume ($\dot{V}$; 13.8 ± 0.9 vs. 12.2 ± 1.0 ml/min; $P < 0.05$), urinary excretion of inorganic phosphate ($U_{Phos}V$) (20.6 ± 2.0 vs. 16.9 ± 2.0 μmol/min; $P < 0.05$) and C_{H_2O} (9.6 ± 0.6 vs. 8.5 ± 0.7 ml/min; $P < 0.05$) were increased following the n-3 fatty acids. There was no difference in fractional free water clearance [(C_{H_2O}/GFR) · 10^2], which averaged 8.7 ± 0.7 during control and 8.7 ± 0.7 following the fish oil supplements. Finally, distal delivery (11.0 ± 1.0 vs. 10.5 ± 0.8) and DFA_{Cl} (0.80 ± 0.04 vs. 0.83 ± 0.01) were not changed with the dietary intervention.

Urinary excretion of PGE_2 in the six female volunteers averaged 168.0 ± 13.0 ng/24 hr and decreased by 26% to 125.3 ± 11.8 ng/24 hr ($P < 0.01$) following the fish oil supplements. Concomitantly, $U_{PGE_3}V$ was hardly detectable during control (6.9 ± 0.8 ng/24 hr) but was increased by the end of the six-week dietary intervention (23.4 ± 3.2 ng/24 hr; $P < 0.01$). Excretion of total PGE (PGE_2 and PGE_3) averaged 174.9 ± 13.5, and was tentatively, however, not significantly, decreased to 148.7 ± 13.4 ng/24 hr by the end of the fish oil supplementation period.

Discussion

The present study demonstrates that, in healthy man, fish oil supplements in the here given dose may be associated with a decrease in renal vascular resistance in the presence of unchanged systemic arterial blood pressure and heart rate. The more pronounced increase in RBF as compared to GFR may

Table 1. Effect of dietary n-3 fatty acid supplements on renal plasma flow (RPF), glomerular filtration rate (GFR), filtration fraction (FF), urine volume ($\dot{V}$) and urinary excretion of sodium ($U_{Na}V$), potassium (U_KV), chloride ($U_{Cl}V$) and inorganic phosphate ($U_{Phos}V$), the clearance of chloride (C_{Cl}), osmolar clearance (C_{Osm}) and free water clearance (C_{H_2O}), fractional free water clearance [$(C_{H_2O}/GFR) \cdot 10^2$], distal fractional chloride absorption [$C_{H_2O}/(C_{H_2O} + C_{Cl})$] and distal delivery [$(C_{H_2O} + C_{Cl})/GFR] \cdot 10^2$

	Control				Following n-3 fatty acids			
	Clearance period				Clearance period			
	I	II	III	Mean ± SEM	I	II	III	Mean ± SEM
RPF *ml/min*	553 ± 48	570 ± 54	559 ± 46	559 ± 45	736 ± 54	733 ± 50	744 ± 51	738 ± 47[c]
GFR *ml/min*	94 ± 7	93 ± 5	102 ± 7	97 ± 3	103 ± 6	108 ± 4	112 ± 6	107 ± 3[b]
FF	0.15 ± 0.01	0.17 ± 0.01	0.19 ± 0.02	0.19 ± 0.01	0.15 ± 0.02	0.16 ± 0.02	0.15 ± 0.01	0.15 ± 0.01[b]
$\dot{V}$ *ml/min*	11.8 ± 1.9	12.9 ± 1.2	12.2 ± 0.8	12.2 ± 1.0	13.6 ± 1.4	14.4 ± 1.0	13.4 ± 1.1	13.8 ± 0.9[a]
$U_{Na}V$ *μmol/min*	287 ± 46	281 ± 51	290 ± 59	284 ± 50	261 ± 34	275 ± 40	289 ± 37	275 ± 36
U_KV *μmol/min*	124 ± 24	144 ± 29	129 ± 21	132 ± 24	122 ± 17	144 ± 19	158 ± 16	152 ± 18
$U_{Cl}V$ *μmol/min*	189 ± 50	226 ± 59	257 ± 60	224 ± 56	177 ± 26	197 ± 34	228 ± 34	201 ± 30
$U_{Phos}V$ *μmol/min*	17.1 ± 2.7	15.3 ± 2.1	16.8 ± 1.7	16.9 ± 2.0	17.2 ± 1.9	22.0 ± 2.1	22.5 ± 2.6	20.6 ± 2.0[a]
C_{Cl} *ml/min*	2.0 ± 0.5	2.2 ± 0.6	2.5 ± 0.6	2.2 ± 0.5	1.7 ± 0.2	1.9 ± 0.3	2.1 ± 0.3	1.9 ± 0.3
C_{Osm} *ml/min*	4.5 ± 0.7	4.2 ± 0.7	4.3 ± 0.5	4.3 ± 0.5	4.5 ± 0.3	4.4 ± 0.4	4.3 ± 0.4	4.4 ± 0.3
C_{H_2O} *ml/min*	8.7 ± 1.0	8.7 ± 1.0	7.9 ± 0.8	8.5 ± 0.7	9.0 ± 1.3	10.0 ± 0.8	9.0 ± 0.8	9.6 ± 0.6[a]
$(C_{H_2O}/GFR) \cdot 10^2$	9.5 ± 1.2	8.3 ± 1.2	7.8 ± 0.7	8.7 ± 0.7	8.9 ± 1.1	9.3 ± 0.6	8.0 ± 0.6	8.7 ± 0.7
$\frac{C_{H_2O}}{C_{H_2O} + C_{Cl}}$	0.82 ± 0.03	0.81 ± 0.04	0.76 ± 0.05	0.80 ± 0.04	0.83 ± 0.02	0.85 ± 0.02	0.80 ± 0.02	0.83 ± 0.01
$\frac{C_{H_2O} + C_{Cl}}{GFR} \cdot 10^2$	11.9 ± 1.6	11.5 ± 1.1	10.0 ± 0.7	11.0 ± 1.0	10.6 ± 1.2	11.1 ± 0.8	10.0 ± 0.8	10.5 ± 0.8

Given are the individual and mean values of the three clearance periods during control and following six weeks of n-3 fatty acid supplements. [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$, as compared to control values

point to a preferential effect of the n-3 fatty acid supplements upon efferent glomerular arteriolar resistance.

The mechanisms of the vasodilatory effect of n-3 fatty acid supplements within the renal circulation remain unclear. Changes in renal eicosanoid metabolism with alterations in the balance of the vasodilatory prostacyclin and the vasoconstrictor thromboxane remain a likely, however unproven, explanation. With respect to intrarenal blood flow, endogenous angiotensin II may act as a vasoconstrictor agent with a more pronounced effect on efferent as compared to afferent renal glomerular arterioles [19]. Moreover, it has recently been shown that dietary fish oil supplements may be associated with a blunted effect of exogenous angiotensin II on systemic arterial blood pressure [2]. It could therefore be speculated that a functional angiotensin II-antagonism within the renal circulation may participate in the renal hemodynamic effect of dietary fisn oil supplements.

In more detail, the increase in GFR observed in the present study points to changes in transcapillary hydraulic pressure and/or the ultrafiltration coefficient, that is, total surface area and/or hydraulic permeability associated with the n-3 fatty acid supplements [20]. At present it remains unclear whether these changes would translate into beneficial effects on renal function in long-term clinical studies. So far, controversial results with n-3 fatty acids have been presented with respect to renal function in a variety of experimental and clinical conditions [21–25]. Our own study points to the fact that renal blood flow and GFR may increase in humans on fish oil supplements. However, these short-term effects should be interpreted cautiously on the basis of the recently established association of increased glomerular pressure, hyperfiltration and the development and progression of renal insufficiency [26]. Therefore, renal function should be monitored in long-term studies, especially if n-3 fatty acids should be more widely used in the therapeutic management of various disorders.

In spite of the marked changes in renal hemodynamics with a pronounced increase in GFR, renal electrolyte excretion was unchanged, suggesting renal tubular effects associated with the fish oil supplements. The precise tubular localization of increased solute reabsorption remains unclear. The clearance techniques used in the present study did not reveal differences with respect to the "distal delivery" term. In the presence of increased GFR unchanged "distal delivery" as calculated in the present study suggests that the proportion of filtered solute reabsorbed proximal to the diluting segment was unchanged. In absolute terms, it thus points to increased amounts of solutes reaching the diluting segments. Increased urine volume and urinary excretion of inorganic phosphate following the dietary n-3 fatty acid supplementation, as observed in the present study under conditions of maximal water diuresis, are compatible with this finding. Consequently, unchanged solute excretion in the presence of increased GFR is therefore most likely due to increased reabsorption in the distal portion of the nephron. This is tentatively supported by the slight increase in C_{H_2O} values following the n-3 fatty acid supplements. With the reservations that these type of clearance studies deserve, it may therefore be concluded that dietary supplementation with n-3 fatty acids

may affect the reabsorptive capacity within the diluting segments of the nephron.

To date, the effect of dietary fish oil supplements on renal PGE metabolism has not been investigated systemically. In the present study, n-3 fatty acid supplements were associated with a significantly decreased urinary excretion rate of PGE_2. The ability of the human kidney to form PGE_3 has been suggested in a study using gas chromatography/mass spectrometry [27]. In that study in three male volunteers, dietary supplements of 40 ml of cod liver oil for four weeks resulted in urinary PGE_3 excretion rates of about 20% those of PGE_2, while no PGE_3 could be detected in the urine of one control person not receiving the n-3 fatty acid supplements. However, the results of this study do not allow the conclusion that PGE_3 is formed in human kidneys since urinary excretion of PGE in males may not exclusively represent renal biosynthesis [28]. Therefore, the demonstration of increased urinary excretion of immunoreactive PGE_3 in six female volunteers in the present study adds further support to the notion that the human kidney may indeed produce significant amounts of PGE_3 during a high intake of n-3 fatty acids. Since PGE_2 may be involved in the regulation of tubular NaCl absorption [29–31], it may be speculated that the quantitative and qualitative changes in PGE biosynthesis could participate, at least in part, in the alterations of tubular electrolyte absorption during a high intake of n-3 fatty acids.

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